AMENDED CLAIMS

[received by the International Bureau on 08 September 2005 (08.09.05),
original claims 4 and 10 amended;
remaining claims unchanged (3 pages)]

+ STATEMENT

1. A method of preparing a recombinant adenovirus (RAdEs) vaccine - The Accession Number 04121701 - to protect against Japanese encephalitis virus (JEV) infection, wherein the said vaccine produces secretory envelop protein (Es) of JEV, said method comprising steps of:
   a. digesting plasmid pMEs with restriction enzymes *Kpn* I and *Bam* HI to obtain cDNA encoding JEV proteins prM and Es,
   b. ligating the cDNA to adenovirus shuttle plasmid pShuttle digested with restriction enzymes *Kpn* I and *Hind* III at the *Kpn* I end,
   c. filling nucleotides at the free *Bam* HI and *Hind* III ends with T4 DNA polymerase to create blunt ends,
   d. ligating the blunt ends together to yield shuttle plasmid pSEs with JEV cDNA encoding the proteins prM and Es,
   e. digesting the shuttle plasmid pSEs with restriction enzymes I-*Ceu* I and Pl-*Sce* I to obtain expression cassette containing the JEV cDNA together with the CMV promoter/enhancer and BGH polyadenylation signal,
   f. ligating the digested shuttle plasmid with I-*Ceu* I and Pl-*Sce* I digested adenovirus plasmid pAdeno-X to generate plasmid pAdEs containing Es expression cassette,
   g. digesting the plasmid pAdEs with *Pac* I,
   h. transfecting the monolayers HEK 293 cells with digested plasmid pAdEs for about one week, and
   i. obtaining the recombinant virus RAdEs vaccine. The Accession Number 04121701.
2. A method as claimed in claim 1, wherein the transfection is at about 37^0C temperature.
3. A method as claimed in claim 1, wherein the JEV proteins are under the control of human CMV IE promoter/enhancer.

4. A recombinant adenovirus (RAdEs) vaccine comprising JEV Es protein prepared by method of claim 1 having Accession No. 04121701, optionally along with pharmaceutically acceptable additives.
5. A vaccine as claimed in claim 4, wherein the vaccine produces secretory envelope protein of JEV.
6. A vaccine as claimed in claim 4, wherein the vaccine protects against Japanese encephalitis virus (JEV) infection.
7. A vaccine as claimed in claim 4, wherein the vaccine is effective by intra-muscular route of administration.
8. A vaccine as claimed in claim 4, wherein the additives are selected from a group comprising alum, gelatin and thiomersal.
9. A plasmid pAdEs of SEQ ID No. 1.
10. Use of a pharmaceutically effective amount of recombinant virus RAdEs vaccine comprising JEV Es protein prepared by method of claim 1 having Accession No. 04121701, optionally along with additive(s) to the subject in need thereof for Japanese encephalitis virus (JEV) infection.
11. Use as claimed in claim 10, wherein the method shows 100% efficacy.
12. Use as claimed in claim 10, wherein the method helps protect subject against encephalitis.
13. Use as claimed in claim 10, wherein the subject is animal.
14. Use as claimed in claim 10, wherein the subject is a human being.
15. Use as claimed in claim 10, wherein the immunization activates both humoral and cell-mediated immune response.
16. Use as claimed in claim 10, wherein the humoral response to the vaccine comprises IgG1 type of antibody.
17. Use as claimed in claim 10, wherein the method leads to high amount of IFN-gamma secretion.
18. Use as claimed in claim 10, wherein immunization leads to moderate levels of IL-5 synthesis.
19. Use as claimed in claim 10, wherein increased amount of RAdEs leads to higher immune response.

20. Use as claimed in claim 10, wherein the method is more effective than the commercially available vaccines.

STATEMENT UNDER ARTICLE 19(1)

The Applicant respectfully submits that the claims are sufficiently amended to overcome the anticipation and obviousness rejections.

Further, the Applicant surprised to notice that International Search Authority has not received a copy of the priority application. We have made a request to the Indian Patent Office to forward the same to International Bureau of WIPO. A copy of the recent request is enclosed herewith for your ready reference. Accordingly, you are requested to acknowledge priority for the instant applicant. In addition, you are respectfully requested to confirm the receipt of the document from Indian Patent Office.

Now, we refer to Item-I of the Written Opinion wherein we hereby state that the information provided in the computer readable form in our letter dated 18.05.2005 to identical to the written sequence listing provided on even date. Thus, the Examiner is respectfully requested to withdraw the requirement.

On the issue of novelty under article 33(2) PCT raised by the Ld. Examiner. The Applicant respectfully submits that claim 4 is amended to incorporate the specifity of JEV Es protein to highlight the novelty aspect of the claim 4. Here, the Applicant would thank the Examiner for acknowledging novelty of the remaining claims 1-3 and 5-20.

Now, in view of the aforementioned amendment in claim 4. We request the Examiner establish novelty for all the claims 1-20.

On the issue of obviousness under Article 33(3) PCT the applicant respectfully submits:

The learned Examiner has rejected the claims 4 to 8 (vaccine *per se*) and 10-20 (use of vaccine) on the basis of obviousness in view of Citations D1 to D3.

The Examiners' contention is that C-terminal truncated E-protein is known to be more immunogenic that anchored E-protein in JEV. Thus, the inventors had sufficient motivation to truncate the E-protein at C-terminal to arrive at a protein and accordingly, prepare a vaccine.

Here, the Applicant respectfully submits that contrary to the observation of the cited arts we found that both the full length (anchored E) and the truncated JEV E protein (secretory) were similar in their antigenicity and protective efficacy in inbred Balb/c mice using the intra-cerebral JEV challenge model (Kaur et al, 2002). Therefore, we had no real motivation to choose the truncated E protein for its perceived enhanced immunogenicity. This is indirect contradiction to the expected results. Thus, it cannot be obvious to a person of ordinary skilled in the art.

We found that the recombinant adenovirus synthesizing the natural sized JEV E protein (that is, the full length) did not grow well in cultured cells and so was likely to be less immunogenic. So, it was actually practical difficulty in growing the cell, which directed us towards the instant invention.

However, the truncated E gene was found to be compatible with our recombinant production system. It turned out to be more immunogenic than the recombinant adenovirus carrying the full length JEV E gene, due to its improved ability to infect cells and thus better gene expression. So we chose the truncated E gene for making stable recombinant that had higher efficiency for infection and thus gene expression. So, it would be inappropriate to consider invention to be obvious when the instant invention is achieved after years of experimental limitations and sophistication.

In fact, the recent commercialization of the technology of the instant application by the Applicant / inventor further establishes the inventiveness of the invention.

Furthermore, we have amended claims 4 and 10 and have made them dependent upon claim 1, pertaining to "method of preparing the vaccine". This amendment would bring in a limitation in the claim 4 pertaining to vaccine *per se* and in addition would make it easier for the Learned Examiner to appreciate the inventiveness of the claim 4.

On the issue of disclosure and support under Article 5 and 6 PCT, the Applicant respectfully submits that the adenovirus of the instant application comprises both prM and Es. Here, the applicant would like to draw the attention of the Examiner to the fact that under step (d) of claim 1, the ligation to yield shuttle plasmid indeed comprises both prM and Es. Furthermore, at a no point of time the protein prM is deleted in the process of claim 1. The only reason "Es protein" is highlighted more throughout the specification including claims is because that is the more critical protein in the adenovirus, required to achieve desired function of the vaccine of the instant application.

Therefore, the Examiner is respectfully requested to withdraw the "disclosure and the support" requirement.

Lastly, as regards the clarity issue of under Article 6 PCT. The Applicant is respectfully submits that the names chosen for various components of the instant application are logical and are not arbitrary in nature. Further, the patents give opportunity to the inventors/applicant to even create new terminologies and popularize the same. Therefore, terminologies like plasmid "pMEs" and recombinant adenovirus vaccine "RAdEs" should be acceptable. Thus, the Examiner is respectfully requested to withdraw the clarity issue.

In view of the fact that all the issues of the combinent International Search Report and the Written Opinion addressed by the Applicant, the Examiner is respectfully requested to withdraw and the rejections and issue a favorable report.